α-Chymotrypsin-catalysed peptide synthesis using activated esters as acyl donors

PERKIN 1 COMMUNICATION

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The coupling efficiency in α-chymotrypsin-catalysed peptide synthesis is greatly improved by the use of activated esters such as the 2,2,2-trifluoroethyl ester as acyl donor instead of the conventional methyl ester; this approach is useful for the incorporation of non-protein amino acids into peptides.

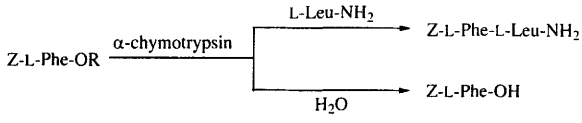
There has been a growing interest in the use of enzymes as catalysts for organic synthesis in recent years. Peptide synthesis using enzymes[1] has come to constitute an important complement to chemical methods and even to recombinant DNA techniques. However, most of the relevant work deals with peptides consisting of only proteinogenic amino acids, though peptides suitably substituted by non-protein amino acids are useful as analogues of biologically active peptides.[2] Accordingly, the enzymatic incorporation of non-protein amino acids into peptides remains a challenge. We have recently undertaken some relevant investigations using easily available proteases such as α-chymotrypsin and papain. The α-chymotrypsin-catalysed couplings of substituted phenylalanines were investigated, employing their methyl esters as acyl donors in acetonitrile containing a small amount of water. We found that the coupling efficiencies were rather low compared to those of the parent phenylalanine as carboxy component. The successful use of 2-chloroethyl and 2,2,2-trifluoroethyl esters in the lipase-catalysed enantioselective hydrolysis or transesterification of amino ester substrates[3] prompted us to try such activated esters as acyl donors instead of the conventional methyl ester in order to overcome this difficulty.

Initially, the α-chymotrypsin-catalysed synthesis of Z-L-Phe-L-Leu-NH_2† was chosen as a model system and several kinds of activated esters were tested as donor esters. The enzyme was adsorbed on Celite and used as a suspension in acetonitrile‡ containing 4% Tris buffer (pH 7.8).[4] The yields of the desired peptide and the hydrolysis product of the donor ester after 1 h of incubation are summarised in Table 1.§ The coupling yield was almost doubled when the acyl donor was changed from the methyl ester to the 2-chloroethyl ester,¶ and the use of the 2,2,2-trifluoroethyl ester was much more effective, forcing the reaction to completion, while the competing hydrolysis of the donor ester was little accelerated compared with the case of the methyl ester. Such a significant increase in the coupling efficiency on replacing the methyl ester with the trifluoroethyl ester was also observed in the couplings with other amine nucleophiles.

Table 1 Effect of donor esters on the α-chymotrypsin-catalysed synthesis of dipeptides[a]

C-Component	N-Component[b]	Yield (%) Peptide	Yield (%) Hydrolysis product[c]
Z-L-Phe-OMe	L-Leu-NH_2	31	1
Z-L-Phe-OCH_2CH_2Cl	L-Leu-NH_2	57	1
Z-L-Phe-OCH_2CCl_3	L-Leu-NH_2	96	3
Z-L-Phe-OCH_2CF_3	L-Leu-NH_2	98	2
Z-L-Phe-$OCH(CF_3)_2$	L-Leu-NH_2	70	7
Z-L-Phe-OMe	Gly-NH_2	63	4
Z-L-Phe-OCH_2CF_3	Gly-NH_2	99	0.6
Z-L-Phe-OMe	L-Val-NH_2	17	2
Z-L-Phe-OCH_2CF_3	L-Val-NH_2	97	3

[a] Coupling conditions as described in the Experimental section, using 0.05 mmol of the C-component and 0.2 mmol of the N-component; reaction time, 1 h. [b] Used in the form of hydrochloride with an equimolar amount of TEA. [c] Z-L-Phe-OH.



This promising method employing the trifluoroethyl ester was applied to the couplings of non-protein amino acids as carboxy components. Thus, esters of *N*-Z-DL-halophenylalanines were allowed to react with L-Ala-NH_2 or L-Leu-NH_2. As shown in Table 2, the peptide yields obtained using methyl esters were rather low; especially noteworthy was the deleterious effect of bulky substituents on the aromatic ring. On the other hand, yields were greatly improved by the use of trifluoroethyl esters without a large increase in the yield of hydrolysis products of the donor esters. In some cases, the reaction proceeded almost to completion. Moreover, it is worth noting that the reactions proceeded diastereospecifically to produce only the L-L peptides even when the trifluoroethyl esters were used.

This approach was also applied to segment condensations. The coupling of Gly-L-Phe or Gly-Gly-L-Phe as carboxy component with L-leucine was fast even when the methyl ester was employed.‖ The coupling yield was greatly increased by the use of the trifluoroethyl ester without accelerating the hydrolysis of the donor ester. In addition, no racemisation accompanied the coupling even when the trifluoroethyl ester was employed.

† Abbreviations: Z, benzyloxycarbonyl; TEA, triethylamine; Phe(2F), 2-fluorophenylalanine; Phe(3F), 3-fluorophenylalanine; Phe(4F), 4-fluorophenylalanine; Phe(2Cl), 2-chlorophenylalanine; Phe(4Cl), 4-chlorophenylalanine; Phe(4Br), 4-bromophenylalanine.

‡ Other hydrophilic organic solvents (propan-1-ol, 2-methylbutan-2-ol, acetone, 1,4-dioxane, tetrahydrofuran, *etc.*) containing a small amount of water were also tested as solvents, and acetonitrile was found to be the best.

§ Non-enzymatic reactions were not detected under the present reaction conditions even when the activated esters were employed.

¶ 2-Chloroethyl esters were used as acyl donors in the incorporation of D-amino acids into peptides mediated by subtilisin in 2-methylbutan-2-ol.[5]

‖ The yields of the desired peptides were as follows: 57% (R = Me), 94% (R = CH_2CF_3) in the coupling Z-Gly-L-Phe-OR + L-Leu-NH_2 after 30 min; 47% (R = Me), 97% (R = CH_2CF_3) in the coupling Z-Gly-Gly-L-Phe-OR + L-Leu-NH_2 after 15 min. The yield of the hydrolysis product of the donor ester was 4% in each case.

Table 2 Effect of donor esters on the α-chymotrypsin-catalysed synthesis of dipeptides containing non-protein amino acids[a]

C-Component	N-Component[b]	Time/h	Yield (%)	
			Peptide[c]	Hydrolysis product[d]
Z-DL-Phe(2F)-OMe	L-Ala-NH_2	5	15[e]	0.1
Z-DL-Phe(2F)-OCH_2CF_3	L-Ala-NH_2	5	47[e]	3
Z-DL-Phe(2F)-OMe	L-Leu-NH_2	1	16[f]	4
Z-DL-Phe(2F)-OCH_2CF_3	L-Leu-NH_2	1	46	2
Z-DL-Phe(3F)-OMe	L-Leu-NH_2	1	15[g]	2
Z-DL-Phe(3F)-OCH_2CF_3	L-Leu-NH_2	1	48	2
Z-DL-Phe(4F)-OMe	L-Ala-NH_2	4	14[e]	0.3
Z-DL-Phe(4F)-OCH_2CF_3	L-Ala-NH_2	4	44[e]	1
Z-DL-Phe(2Cl)-OMe	L-Ala-NH_2	2.5	3[e]	0
Z-DL-Phe(2Cl)-OCH_2CF_3	L-Ala-NH_2	2.5	42[e]	0
Z-DL-Phe(4Cl)-OMe	L-Leu-NH_2	1	4[h]	1
Z-DL-Phe(4Cl)-OCH_2CF_3	L-Leu-NH_2	1	48	2
Z-DL-Phe(4Br)-OMe	L-Leu-NH_2	1	1.4[i]	0.2
Z-DL-Phe(4Br)-OCH_2CF_3	L-Leu-NH_2	1	40	2

[a] Coupling conditions: see the Experimental section. [b] Used in the form of hydrochloride with an equimolar amount of TEA. [c] Based on the amount of the racemic C-component. [d] Z-Phe(X)-OH. [e] % L-L = 100. [f] 33% after 5 h. [g] 38% after 5 h. [h] 12% after 5 h. [i] 6% after 5 h.

The results obtained so far indicate the usefulness of the method using activated esters such as the 2,2,2-trifluoroethyl ester as acyl donors. Since there seems to be no significant difference in the binding of the donor ester to the enzyme between the methyl and trifluoroethyl esters, the rates of acylation of the enzyme may be very different between them. The use of the activated ester must accelerate the acylation step. Further studies are needed to clarify the scope and limitations of this approach.

Experimental

The enzyme adsorbed on Celite was prepared as follows: α-chymotrypsin (Sigma Type II, *ex* bovine pancreas) (32 mg) dissolved in Tris buffer (pH 7.8) (4 cm^3) was mixed with Celite No. 535 (1 g), and the mixture was lyophilised.

α-Chymotrypsin-catalysed peptide synthesis

The coupling of Z-DL-Phe(2F)-OCH_2CF_3 with L-Ala-NH_2 is described as a typical example. A mixture of Z-DL-Phe(2F)-OCH_2CF_3 (0.1 mmol), L-Ala-NH_2·HCl (0.2 mmol), TEA (0.2 mmol) and the immobilized enzyme on Celite (150 mg, corresponding to 4.6 mg of α-chymotrypsin) was incubated with shaking in a solvent composed of acetonitrile (2 cm^3) and Tris buffer (pH 7.8) (8×10^{-2} cm^3) at 30 °C. The amounts of the donor ester, peptide and hydrolysis product were determined by HPLC analysis using an ODS column (mobile phase, 65% MeOH aq. containing 0.01 M H_3PO_4; flow rate, 1.0 cm^3 min^{-1}; column temperature, 30 °C; detection, UV at 254 nm). The diastereomers (L-L and D-L) of the resulting peptides were also separated well by decreasing the amount of MeOH in the above mobile phase.

Acknowledgements

This work was supported in part by grants from the Science Research Promotion Fund of the Japan Private School Promotion Foundation and from the Ministry of Education, Science and Culture, Japan (to T. M.)

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Paper 6/06546E
Received 23rd *September* 1996
Accepted 23rd *October* 1996